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Evolution of plasma trace element status in children treated with low-protein diets related to Maple Syrup Urine Disease (MSUD) and Urea Cycle Disorder (UCD)

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ABSTRACT

The main treatment for Urea Cycle Disorder (UCD) and Maple syrup urine disease (MSUD) is low-protein diets and element deficiencies may develop related to dietary therapy. This study aimed to determine plasma trace element levels in patients with MSUD and UCD and to compare these results with those established in a healthy pediatric population not undergoing any dietary treatment. The study was conducted with 30 patients diagnosed with MSUD and UCD, and a control group of 30 health age-matched children. Dietary treatment involved a natural protein-restricted diet supplemented with a special formula, depending on the specific metabolic defect. Significant differences were observed between patients and control values for magnesium, selenium, and copper ($p<0.05$). These results reinforce that patients under dietary treatment should be regularly monitored for these elements. Furthermore, fortifying these elements in special formulas (as they are the sources of essential amino acids) should be considered.

Keywords: trace element, children, MSUD, UCD, protein-restricted diet

INTRODUCTION

MSUD is an autosomal recessive inherited metabolic disorder (IMD) caused by a deficiency in branched-chain α-ketoacid dehydrogenase (BCKD), resulting in the

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accumulation of branched-chain amino acids (BCAAs) such as leucine (LEU), isoleucine (ILE), and valine (VAL), along with their corresponding α-ketoacids (BCKAs)[1]. The increase in BCAAs prevents the transport of large neutral amino acids to the brain and leads to increases in neurotransmitter synthesis and myelination[2]. MSUD has had a worldwide incidence, occurring in one in 185,000 live births[3]. Although the exact incidence in Türkiye is not known, as MSUD is not currently included in the newborn screening program, it is estimated to affect as many as 1 in 50,000 newborns, particularly due to the high rate of consanguineous marriages[4].

MSUD treatment involves dietary leucine restriction, BCAA-free medical foods, moderate supplementation with isoleucine and valine, and regular clinical and biochemical monitoring[1]. Nutrition therapy plays a crucial role in restoring and maintaining metabolic homeostasis in MSUD[5]. In the diet, the most toxic effect is primarily caused by leucine. Therefore, priority is given to leucine content in the nutrition plan. When leucine is provided, usually isoleucine and valine are also provided. In cases where they cannot be provided, supplements are given[3]. In the diet, energy intake is provided from fruits (<30 mg leucine/100 g and 0.3-1 g protein/100 g) and vegetables (<100 mg leucine/100 g) with low leucine content, commercially available products with low leucine content (≤0.5 g protein/100 g), sugar, liquid oil, and starch[6].

Urea cycle disorders (UCDs) are congenital metabolic errors resulting from defects in one of the six enzymes or two transporters involved in the detoxification of ammonia into urea, which is excreted in the urine[7]. The overall incidence of urea cycle disorders occurs in approximately 1:35.000 births and all, except for OTC deficiency, are autosomal recessive. Loss of function of the urea cycle causes the problem of inability to excrete ammonium produced during protein catabolism. The resulting hyperammonemia is harmful to the brain[8].

The maintenance dietary treatment of UCD involves providing a low-protein diet, supplementing with essential amino acids as necessary, and offering appropriate nutritional support to prevent catabolic stress[9].

Despite this supplemented diet, patients with IMDs on low-protein diets are at risk of deficiency in several micronutrients. Deficiencies in selenium especially in phenylketonuria have been reported[10,11]. Selenium deficiency was also reported in patients with UCD, despite their selenium intake being higher than the Recommended Daily Allowance (RDA)[12]. These findings suggest a potential issue of reduced selenium bioavailability in IMDs. In addition, deficiencies in various micronutrients have been reported in patients with MSUD due to protein restriction. In a case report, skin lesions related to zinc deficiency were noted in an infant diagnosed with MSUD[13].

The objective of this study was to compare plasma levels of cobalt, copper, zinc, selenium, manganese, molybdenum, and magnesium between children undergoing low-protein dietary treatment and their age-matched healthy children without any dietary treatment. The aim was to explore potential deficiencies and their etiology, as well as to assess their association with nutritional status.

METHODOLOGY

Study design and sampling

The study was conducted between January 2015 and May 2017 in Istanbul University Cerrahpasa Medical Faculty. The patient group consisted of individuals undergoing protein-restricted dietary treatment; 19 had MSUD, and 11 had UCD, all of whom were being followed up by the Pediatric Nutrition and Metabolism Department. Inclusion criteria were: being under 18 years of age, undergoing low-protein nutrition therapy due to congenital protein metabolism disorders, attending regular check-ups, having good dietary compliance, and not having malabsorption. Exclusion criteria were: being under bad metabolic control, having malabsorption, not attending regular check-ups, and having poor dietary compliance. Results from these patients were compared with control values established in 30 healthy children who applied to the Healthy Child Polyclinic. Exclusion criteria for control group were: the existence of chronic or acute disease, pharmacological treatments, the use of food supplements containing trace elements, and special diets.

Dietary treatment

Dietary treatment involves a natural protein-restricted diet supplemented with a special formula containing different amino acids, vitamins and trace elements depending on the patient's specific metabolic disorder and age. Participants were asked to complete 3-day food diaries before blood samples were taken for monitoring trace elements. Food records were analyzed using the Nutrition Information System (BEBIS) program to quantify the average nutrient intake[14].

Biochemical analysis

Five milliliters of venous blood were drawn after an overnight fasting and blood was centrifuged for 10 minutes at 3000 rpm. The plasma was isolated and preserved in covered lithium heparin tubes, then stored at -20°C until the measurement of trace elements was conducted. Plasma levels of cobalt, copper, zinc, selenium, manganese, molybdenum, and magnesium were examined utilizing ICP-MS (Inductively Coupled Plasma – Mass Spectrometry) at the Forensic

Toxicology Laboratory of Istanbul University Cerrahpasa Faculty of Medicine, Forensic Medicine Institute. The study protocol strictly adhered to all relevant national regulations, institutional policies, and ethical principles outlined in the Helsinki Declaration. Approval was secured from the Ethical Committee of Cerrahpasa Medical Faculty (01.07.2014/No:83045809/604.01.01), and informed consent was obtained from the parents of all participating patients.

Statistical analysis

Descriptive statistics including mean, standard deviation, median, minimum, and maximum values, as well as frequency and percentage, were utilized in the data analysis. The distribution of variables was assessed using the Kolmogorov-Smirnov test. For the analysis of quantitative independent variables, the Mann-Whitney U test was employed, while the Chi-square test was used for qualitative independent variables. Statistical analyses were performed by using Statistical Package for Social Sciences version 22.0 (SPSS Inc., Chicago, IL, USA).

RESULTS and DISCUSSION

The characteristics of the diet group and control group included in the study are shown in Table 1. There was no significant difference ($p>0.05$) in the ages and gender distribution of patients in the diet and control groups.

Table 1. Age and gender distributions of patients in the diet and control groups

		Control Group (n=30)	Diet Group (n=30)	P
Age	Mean ± SD Median	7.0 ± 4.6 7.0	5.7 ± 5.0 5.0	0.312 [m]
Gender	Male (n, %) Female (n, %)	14 (%46.7) 16 (%53.3)	18 (%60) 12 (%40)	0.301 [x^2]

*m= Mann Whitney U test, x^2= chi-square test

Table 2 shows the concentration of plasma Mg, Mn, Co, Cu, Zn, Se and Mo in the diet and control groups. Statistical analysis showed that the diet group was significantly lower than the control group with regards to Mg, Cu, and Se levels ($p=0.008$, $p=0.001$, and $p<0.001$, respectively). Moreover, no significant differences in Mn, Co, Zn, or Mo levels were observed in the diet group, as compared to the control group ($p>0.05$) (Table 2).

Table 2. Comparison of plasma trace element concentration in diet and control groups

	Control Group (n=30)		Diet Group (n=30)			
	Mean ± SD	Median	Mean ± SD	Median	P	
Mg (x10⁴) (µg/L)	1.3 ± 0.2	1.4	1.2 ± 0.2	1.2	0.008	m
Mn (µg/L)	9.9 ± 10.2	6.3	7.8 ± 5.8	6.5	0.918	m
Co (µg/L)	1.0 ± 1.1	0.7	0.8 ± 0.3	0.7	0.482	m
Cu (µg/L)	1162.0 ± 217.6	1170.0	970.1 ± 192.4	945.9	0.001	m
Zn (µg/L)	607.4 ± 92.5	623.1	648.8 ± 158.5	630.1	0.294	m
Se (µg/L)	64.8 ± 23.1	61.8	26.4 ± 35.4	13.3	0.000	m
Mo (µg/L)	0.2 ± 0.7	0.1	1.0 ± 2.7	0.1	0.281	m

*m= Mann Whitney U test

Average daily oral nutrient intake and energy were calculated for patients with dietary treatment and control group (Table 3). Since the natural protein-restricted diet is a common treatment for these disorders, significant differences were observed between the values of IMDs and control groups compared to natural protein intake ($p<0.05$). In the IMDs and control groups, there was no significant difference in daily energy intake ($p>0.05$). Fat consumption in the IMDs group was significantly lower than in the control group ($p<0.05$). The carbohydrate consumption in the IMDs group was significantly higher than that in the control group ($p<0.05$).

In the IMD group and the control group, there was no significant difference in the dietary intake of magnesium ($p>0.05$) (Table 3). However, the plasma magnesium level of the IMD group was found to be significantly lower than that of the control group ($p<0.05$) (Table 3). For iron and zinc, the daily intake of patients with dietary treatment and the control group (iron: 13.5 ± 6.6 mg/day, 9.4 ± 4.0 mg/day; zinc: 8.5 ± 5 mg/day, 8 ± 2.9 mg/day, respectively) was slightly higher than the recommended daily allowances (RDAs) (iron: 7-11 mg/day; zinc: 4.3-7.4 mg/day, respectively) (Table 3).

Table 3. Comparison of energy and nutrient intake with diet in the IMDs and control groups

	Control Group (n=30)		IMDs Group (n=30)			
	Mean ± SD	Median	Mean ± SD	Median	P	
Energy (kcal)	1443.1 ± 367	1498.9	1396.6 ± 478.4	1336.5	0.836	m
Natural protein (gram)	55.6 ± 18.7	56.0	4.9 ± 2.0	4.5	0.000	m
Fat (gram)	68.7 ± 20.4	70.8	56.9 ± 17.8	57.7	0.025	m
Carbohydrate (gram)	146.4 ± 45.4	144.5	185.8 ± 74.1	192.6	0.028	m
Mg (mg)	213.1 ± 73.5	202.8	195.9 ± 107.0	184.2	0.132	m
Fe (mg)	9.4 ± 4.0	8.6	13.5 ± 6.6	12.5	0.009	m
Zn (mg)	8.0 ± 2.9	8.1	8.5 ± 5.0	7.1	0.416	m

*m= Mann Whitney U test

Results of different elements according to the type of disease in IMD patients under dietary treatment are shown in Table 4. Plasma Mg, Mn, Co, Cu, Zn, and Mo values did not differ significantly between the MSUD and UCD groups ($p>0.05$). However, in the UCD group, plasma Se value was significantly lower than in the MSUD group ($p<0.05$) (Table 4).

Table 4. Comparison of plasma trace element amounts in MSUD and UCD groups.

	Control Group (n=30)		IMDs Group (n=30)			
	Mean ± SD	Median	Mean ± SD	Median	P	
Energy (kcal)	1443.1 ± 367	1498.9	1396.6 ± 478.4	1336.5	0.836	m
Natural protein (gram)	55.6 ± 18.7	56.0	4.9 ± 2.0	4.5	0.000	m
Fat (gram)	68.7 ± 20.4	70.8	56.9 ± 17.8	57.7	0.025	m
Carbohydrate (gram)	146.4 ± 45.4	144.5	185.8 ± 74.1	192.6	0.028	m
Mg (mg)	213.1 ± 73.5	202.8	195.9 ± 107.0	184.2	0.132	m
Fe (mg)	9.4 ± 4.0	8.6	13.5 ± 6.6	12.5	0.009	m
Zn (mg)	8.0 ± 2.9	8.1	8.5 ± 5.0	7.1	0.416	m

*m= Mann Whitney U test

The amounts of energy, fat, magnesium, and zinc intake through diet did not show significant differences between the MSUD and UCD groups (p>0.05). However, in the UCD group, the intake of natural protein, essential amino acids, and iron through the diet was significantly lower compared to the MSUD group (p<0.05) (Table 5).

Table 5. Comparing the amounts of energy and nutrient intake in diets of MSUD and UCD groups

	MSUD (n=19)		UCD (n=11)			
	Mean ± SD	Median	Mean ± SD	Median	P	
Energy (kcal)	1498.3 ± 538.6	1711.0	122 0 ± 296.7	1266.0	0.067	m
Natural Protein (gram)	4.3 ± 1.9	4.1	6.1 ± 1.7	6.0	***0.010***	m
Essential amino acids (gram)	36.8 ± 22.5	35.6	9.1 ± 1.9	9.1	***0.000***	m
Fat (gram)	57.4 ± 18.9	59.2	56.0 ± 16.3	53.8	0.651	m
Carbohydrate (gram)	198.2 ± 85.5	213.5	164.3 ± 44.1	163.8	0.168	m
Mg (mg)	219.3 ± 126.6	193.0	155.4 ± 38.6	134.8	0.168	m
Fe (mg)	15.5 ± 7.4	14.6	10.1 ± 2.9	10.0	***0.027***	m
Zn (mg)	9.7 ± 5.9	7.5	6.3 ± 1.6	6.0	***0.077***	m

*m= Mann Whitney U test

Since a natural protein-restricted diet is frequently employed in the treatment of IMDs, patients with these disorders may experience deficiencies in vitamins and trace elements[15]. Trace elements play a crucial role in metabolic processes and oxidation-reduction reactions within the central nervous system, potentially influencing cognitive function. The treatment of IEMs involves restricting dietary intake of natural protein, which is balanced with a special formula fortified with trace elements, mineral salts, and vitamins[16]. The present study aimed to investigate the levels of trace elements (Zn, Cu, Mn, Se, Co, Mg, Mo) in children with MSUD and UCD, as well as in their age-matched healthy children without dietary intervention.

Previous studies have noted deficiencies in certain trace elements among individuals with IMDs, particularly those with phenylketonuria (PKU)[15,17].

This study found plasma selenium levels were significantly lower in the diet group than in the control group (p<0.05). Similar to this report, Tondo (2010) found significant differences in selenium levels between IMDs ongoing dietary

treatment and control subjects[12]. The study revealed that the average dietary selenium intake among PKU patients exceeded RDA values slightly. However, selenium deficiency was also evident in patients with UCD and organic acidurias, despite their selenium intake surpassing RDA levels[12]. These findings suggest a potential issue of reduced selenium bioavailability in both PKU and other IMD patients, as their average selenium intake exceeded the RDAs. In some studies, selenium deficiency has been reported, associated with decreased selenium intake[10] which has also been documented in patients with PKU due to low-protein diet therapy[11,15].

Van Bakel et al. observed significantly lower selenium levels in plasma samples from children with phenylketonuria compared to healthy children[17]. Unlike the current study and other studies in the literature, no significant differences were observed in plasma selenium levels among the PKU and mild hyperphenylalaninemia (m-HPA) patients and the control group[18].

Selenium deficiencies appear significant due to its involvement in numerous antioxidant metabolic pathways and hormone metabolism[17]. Reduced antioxidant capacity was observed in individuals with PKU, prompting consideration of selenium supplements in relation to oxidative stress parameters[19]. Selenium deficiency has been suggested to be associated with impaired antioxidant function and alterations in thyroid hormone levels[20-23].

Treated PKU patients exhibited decreased glutathione peroxidase activity due to low plasma selenium levels, leading to oxidative stress[24]. Furthermore, DNA damage has been reported, indicating elevated production of reactive species[25-27]. This condition has been linked to increased free radical generation and reduced levels of antioxidant micronutrients[28,29]. These findings resulted in adding selenium, vitamins, and oligo elements to phenylalanine-free mixtures for PKU[30]. Additionally, selenium deficiency in PKU patients has been associated with some impaired cognitive functions[31].

Some studies have associated oxidative stress with zinc deficiency[32] and selenium deficiency[20,21, 33]. Sitta and colleagues demonstrated that long-term supplementation of selenium and carnitine can rectify protein and lipid oxidative damage and reinstate glutathione peroxidase activity[19].

In the current study, plasma copper levels were found to be significantly lower in IMD patients compared to the control group. This contrasts with the findings of Gropper et al., who demonstrated no difference in plasma copper levels between children with PKU (19.2 μmol/L) and the control group (18.7 μmol/L)[34].

The other element that showed significantly lower values in IMD patients under dietary treatment was Mg. Nevertheless, no significant difference was observed in the dietary intake of magnesium between the IMDs group and the control group ($p>0.05$). This result can be attributed to the redistribution of mineral metabolic pools and disruption in intestinal absorption.

A study conducted with PKU patients concluded that exhibits interactive associations with PKU, and serum magnesium levels decrease in PKU patients. Accordingly, this reduction in serum Mg levels in PKU patients may stem from decreased tubular reabsorption and increased urinary excretion[35].

To our knowledge, apart from Tondo's study[12], no studies have been carried out on trace element status in patients with MSUD and UCD. The major limitation of this study, is the correlation between nutrient intake and status is intricate, influenced by factors like nutrient bioavailability, interactions, and individual metabolism. Another limitation is that throughout the study, advancements in markers of nutritional status have been identified. For instance, while all studies reported plasma selenium concentrations as the status indicator, more recently, plasma selenoprotein P (SEPP1) has been regarded as a more informative marker of status. Since studies on this subject are generally conducted in children with PKU, there is not enough data in the literature on trace element levels in patients with UCD and MSUD.

In conclusion, patients with IMDs on protein-restricted diets are at risk of deficiency in magnesium, selenium, and copper. Due to the restricted intake of natural protein, the MSUD and UCD formulas provide a high percentage of the daily requirements for micronutrients. All these results show the importance of evaluating trace element intake levels in the nutritional follow-up of such patients and the necessity of supplementing these trace elements in case of deficiency. Additionally, there is a need to enrich formulas with trace elements, which serve as essential amino acid sources.

STATEMENT OF ETHICS

Our study was approved by Istanbul University Cerrahpasa Medical Faculty's local ethics committee (Ethical approval no: 83045809/604.01.01, Date: 01.07.2014).

CONFLICT OF INTEREST STATEMENT

The authors declared no conflict of interest.

AUTHOR CONTRIBUTIONS

The authors contributed to the work equally.

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